

COMPARATIVE ECOPHYSIOLOGY OF TWO HIGH-ELEVATION SOUTHERN
APPALACHIAN CONIFERS: THE IMPORTANCE OF THE WINTER SEASON

by

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Abstract

Southern Appalachian spruce-fir forests are glacial relicts, located as disjunct “islands in the sky” on the region’s highest peaks. Fraser fir (*Abies fraseri*), a rare endemic and valuable Christmas tree, dominates at elevations above 1650 m while red spruce (*Picea rubens*) occurs at lower elevations (1380 – 1650 m). Winter in this forest type brings extended periods of snowfall and freezing temperatures but is not as severe as in northern boreal regions. Compared to similar forests in the northern latitudes, southern spruce-fir forests have longer winter photoperiods, more frequent midwinter thaws, and fewer, less severe subzero temperatures. Despite the relatively milder winter, this season still constitutes a substantial portion of the annual cycle for these two dominant conifers. However, its importance to the ecophysiology of these trees is poorly understood, as are the potential impacts of milder winter conditions resulting from future climate warming. Warming may result in more frequent occurrences of mild temperatures, shortened durations of thaws, free soil and stem (capacitance) water for uptake by trees, stimulate below- and above ground respiration, and enhance carbon uptake via photosynthesis. My study aims to address this knowledge gap by measuring the comparative ecophysiology of both species during winter on Grandfather Mountain, NC, including pigment concentrations, photosynthesis/respiration, fluorescence, and twig water potentials. Pigment concentrations (per gdw) were significantly higher in *A. fraseri* than in *P. rubens*, and concentrations declined with age in both species; no effect was found for canopy face (N or S). Photosynthetic rate was significantly higher in the one-year-old needles of *P. rubens* on day 4. All other rates of photosynthesis did not differ by needle age. Chlorophyll

fluorescence (F_v/F_m) did not differ between the two species but increased significantly with air temperature, and photoinhibition was higher on cold days and lower on warm days as expected. Water potentials in *A. fraseri* were significantly more negative on two cold days. The lower water potentials in *A. fraseri* may be a result of its relatively higher needle density in a similar size twig (i.e. more demand for water) and may also indicate that its stomata have a lower temperature threshold for opening than do those for *P. rubens*. The results of this study suggest that while net photosynthesis for both species is at or near zero on cold days, the strategies by which they maintain compensation may be different and could have an influence on their relative ability to take advantage of midwinter thaws. Further exploration of differences in hydraulic conductivity, pigment cycling, and conduit diameter is necessary to conclude the underlying causes of the differential wintertime activity.

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Dedication

This thesis is dedicated to Sara Jordan, Jessie Jordan, and Tori Nothnagel for their unconditional love and support. It is also dedicated to Zane Sink and Jessica Stevens, for showing me what it means to be a truly passionate scientist, and to the arborists, tree farmers, and foresters who have shared their skills, passion, and perspectives with me.

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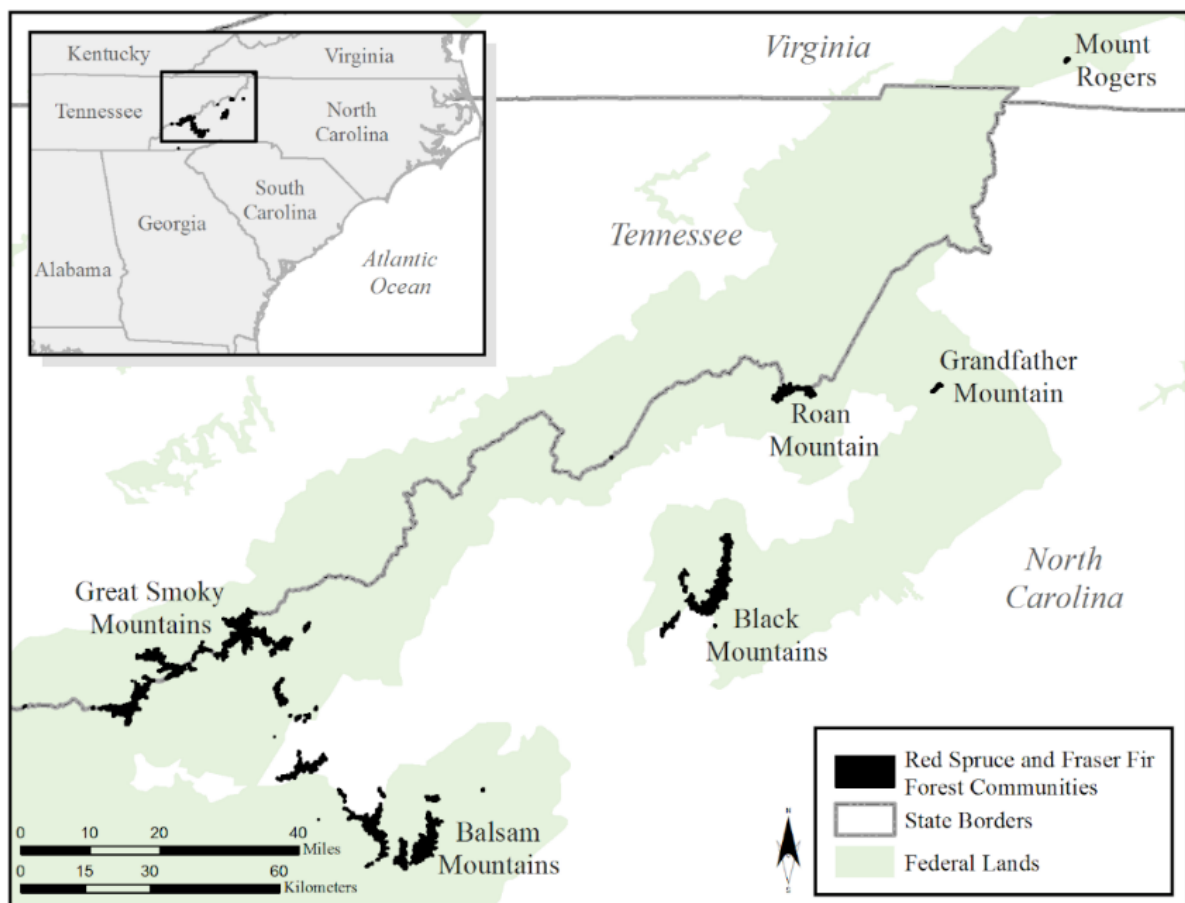
Introduction

The southern Appalachian spruce-fir forest is restricted to small, disjunct populations in the highest peaks of the Appalachian Mountains of North Carolina, Tennessee, and Virginia (Figure 1.1). This forest type is a glacial relict from the last ice age when temperatures were much colder, but today it consists only of stranded spruce-fir communities—or “islands in the sky”—where the climate remains mild enough for the trees to persist. The threat of climate change in this rare, sensitive ecosystem has led to it being labeled the second most endangered ecosystem in North America (Heinz 2002).

Reminiscent of the boreal forests in the northern latitudes, this forest is characterized by mild, cool summers and extended, cold winters. However, conifers in the southern Appalachians are exposed to different environmental conditions than those in boreal forests. Southern spruce-fir (SSF) forests have longer winter photoperiods (i.e. length of day) than northern boreal (NB) forest ecosystems, while the converse is true during the summer. SSF forests reach maximum summer temperatures of about 22 °C, while lower elevation NB forests are subject to wider temperature ranges due to continental heating; winter temperatures are typically much lower in NB forests, and soils remain frozen for longer periods of time (Cogbill and White 1992). Montane ecosystems like the SSF forest experience higher yearly amounts of rainfall throughout the year and are also frequently immersed in fog (about 65% of the year), resulting in a more consistently moist environment (Berry and Smith 2012).

Rainfall and fog are plentiful in the SSF forest, but snowfall is inconsistent (mostly depending on elevation) with snowpack largely absent at the lower-elevation sites. While

higher elevation SSF ecosystems can experience anywhere from 250-400 cm of snowfall in a single season as well as constant snow cover, snowpack still does not remain consistent among all high elevation sites, likely owing to differences in exposure, i.e. canopy openness (Martin et al. 2015). Although NB and SSF forests are dominated by the same genera of conifers, the climatic conditions unique to the SSF forest—especially those in the winter—may produce ecophysiological responses distinct from those of closely related NB conifers.



Andi M. Sigsbey
7/16/2013

Figure 1.1. Distribution of southern Appalachian spruce-fir forests. From White, van de Gevel, and Soulé (2012).

As its name suggests, the SSF forest is characterized by two conifers, the Fraser fir (*Abies fraseri* (Pursh) Poir.), which occurs in pure stands at higher elevations (>1523 m) and red spruce (*Picea rubens* Sarg.), which persists at lower elevations and is much more widespread (Figures 1.2 and 1.3). The Fraser fir is a moderately sized tree (maximum height 26 meters) with a relatively short lifespan of 75-100 years; red spruce, on the other hand, can grow much taller (up to 35 m in the SSF), with some trees living up to 400 years (Burns and Honkala 1990). Fraser firs have a needle retention period of around 3-4 years, whereas red spruce retains its needles for much longer; moreover, species in the *Picea* genus appear to have longer needle retention periods in montane ecosystems than in lower elevation habitats (Reich et al. 1996).

Why Fraser firs dominate exclusively at the highest elevations of the southern Appalachians has never been formally studied, nor have the physiological mechanisms driving the differential distributions of Fraser fir and red spruce been elucidated. Researchers have postulated a variety of potential mechanisms that might explain the different distributions of these two species, among which are greater cold tolerance in Fraser fir; greater competitive ability of spruce and hardwoods at lower elevations (which prevent the fir from establishing there); and the ability to persist at the highest elevations by achieving positive annual carbon gain, in part due to the lack of water stress from the high rainfall amounts and frequent fogs that greatly reduce water loss from the trees.

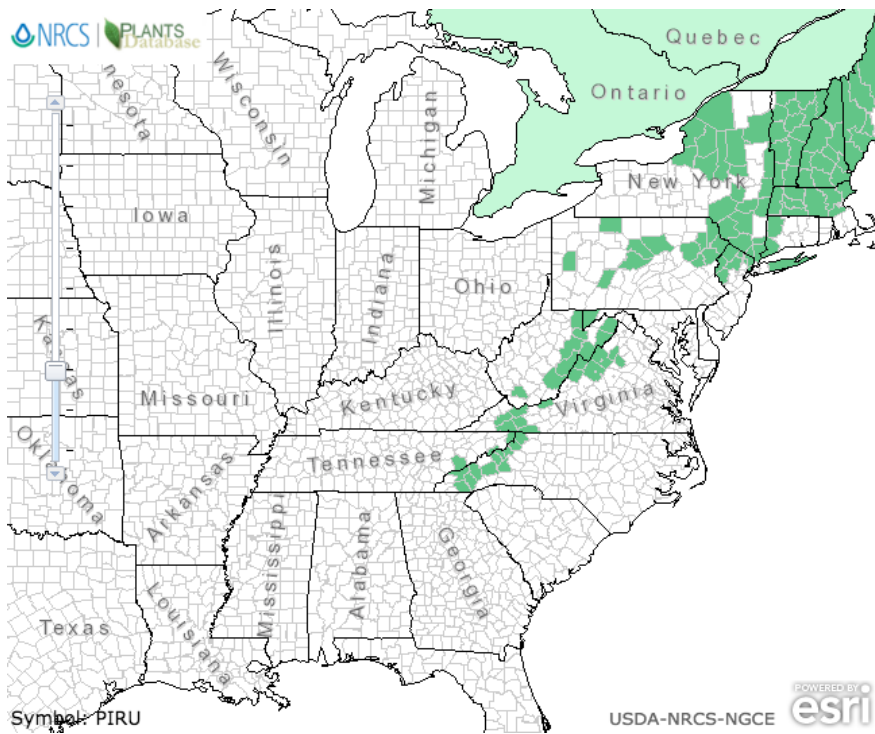


Figure 1.2. Native distribution of red spruce (*Picea rubens*). From USDA Plants Database.

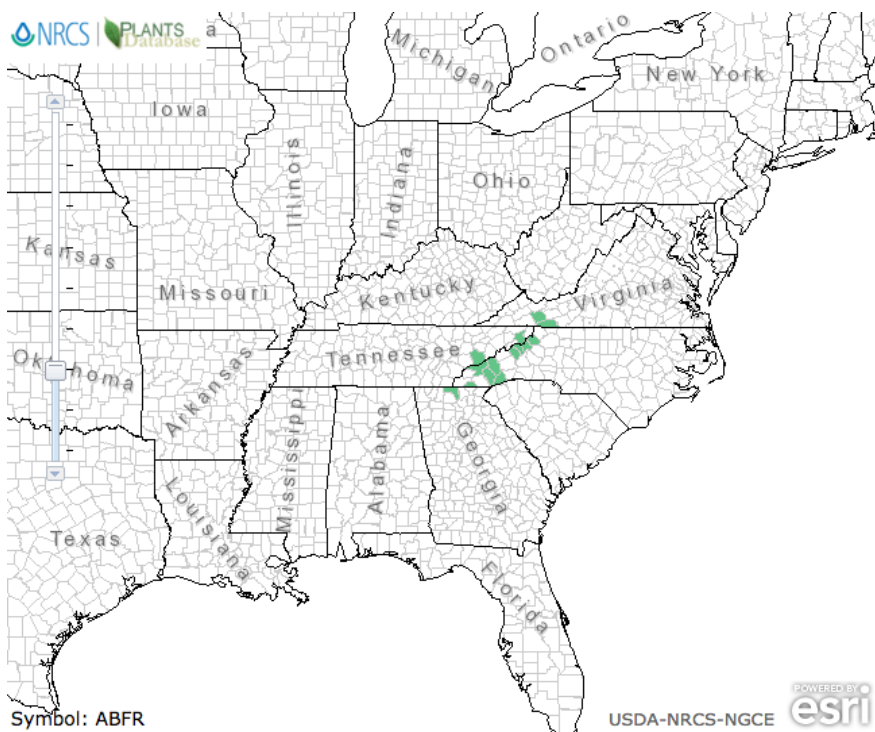


Figure 1.3. Native distribution of Fraser fir (*Abies fraseri*). From USDA Plants Database.

While natural factors certainly influence the makeup of the SSF forest, humans may have also played a role in the formation of the visibly sharp transition from spruce-fir forest to pure stands of Fraser fir. Extensive logging could have significantly affected present-day species distributions, as nearly all stands of red spruce and Fraser fir in the southern Appalachians have been either clear-cut or extensively logged. As result, the current distribution of Fraser fir and red spruce along the elevational gradient may be more indicative of their respective ability to re-colonize open spaces than their true capacity to persist at a particular elevation (White et al. 2014). Although the mechanisms underlying the distribution difference should be interpreted with caution, current occurrence combined with physiological study can still be a useful tool for projecting future response to a changing climate.

The rare, endemic Fraser fir is found on just seven mountaintops in the wild. However, it is grown commercially beyond this small range at elevations as low as 670 m. It appears that Fraser firs are ecophysiologicaly flexible, as demonstrated by the fact that they can flourish on farms at elevations far below the cloud ceiling when protected from pests and competitors. Recent studies have demonstrated physiological plasticity in Fraser firs in these settings, showing no significant differences in ^{13}C discrimination or overall growth of trees of the same age along an elevational gradient (Stevens 2017; Z.R. Sink, R.K. Jordan, and H.S. Neufeld, unpubl. data). Another study on farm-grown Fraser firs suggested possible benefits to most farmers, excepting those at the very lowest farm elevations, due to a longer growing season (Cory 2015).

Cloud immersion periods have been shown to increase the rate of photosynthesis, or assimilation (A), in Fraser fir. This is accomplished due to increasing constant diffuse irradiance (a more even distribution of light on all sides of the tree); decreasing evapotranspiration (less water lost from the tree); improving water relations (more available water means less water stress); and increasing temperature homogeneity throughout the crown of individual trees (Berry and Smith 2012; Berry and Smith 2013; Hernandez-Moreno et al. 2017; Johnson and Smith 2006; Reinhardt and Smith 2008).

While Fraser firs benefit from cloud immersion, it is less clear if red spruce receives the same benefits. Although red spruce has similar rates of foliar water uptake during periods of immersion, the Fraser fir seems to derive more of its total foliar water content from fog than the red spruce (Bowden et al. 1989; Boyce et al. 1991; Berry and Smith 2014). This difference could be influenced by different needle packing density and retention periods, different permeability of the leaf cuticle due to thickness and/or propensity for cracking, or simply more frequent exposure to fogs, which increase with increasing elevation in the SSF forest. While immersion does not fully explain the differential distributions of Fraser fir and red spruce, the Fraser fir appears more sensitive to immersion, which suggests potentially different responses to a rising cloud ceiling.

The flexibility and success of the Fraser fir on farms well below its native elevation suggest that it is more tolerant of heat and drought than would be inferred from its natural distribution. However, the general research focus on summer warming has resulted in virtually no winter ecophysiological data for the Fraser fir, leaving a significant gap in the understanding of its physiological capacity or potential response to winter warming.

Unlike the Fraser fir, red spruce has been the subject of winter ecophysiological study for decades. This is likely due to its commercial value and relative abundance, as red spruce is found in both montane and boreal eastern spruce-fir forests extending northward into Canada (White and Cogbill 1992). While red spruce is a valuable timber tree in other areas, it currently has limited commercial value in the southern Appalachians due to previous overharvesting of native stands (White et al. 2012). While its ecophysiology has been studied more than the Fraser fir, few studies have focused specifically on winter activity of red spruce in the unique climate of the southern Appalachian region.

Most of the studies relating to the winter ecophysiology of red spruce are related to the widespread spruce decline seen in the 1980s through the 1990s. Decreased cold hardiness related to air pollution was found to be a contributing factor in the marked decline of red spruce populations, exacerbated by the existing physiology of the red spruce itself (Friedland et al. 1984). Red spruce has a well-documented, somewhat counterproductive ability to rapidly respond to midwinter thaws at the expense of its protection from cold injury (Schaberg 2000; DeHayes et al. 2001). This response, referred to as dehardening, is triggered in evergreen plants by warming temperatures such as in the winter-spring transition. However, a sufficiently long midwinter thaw event with both days and nights above freezing temperatures can imitate the advent of spring, triggering premature dehardening and causing extensive foliar damage (particularly current year needles) in subsequent freezing events (Schaberg 1996).

Red spruce appears especially vulnerable to frost injury even when compared to other trees in the same forest. Strimbeck et al. (1995) found that in a northern spruce-fir

forest containing red spruce and balsam fir (*Abies balsamea*, the closest relative of the Fraser fir), red spruce exhibited significant dehardening of as much as 14 °C within three days; dehardening was undetectable in balsam fir under the same conditions. By contrast, the re-hardening duration for red spruce took 20 days, during which substantial frost injury was incurred. The thaw responses from balsam fir and red spruce may suggest a similar difference between Fraser fir and red spruce, potentially shedding light on their distributional difference in SSF forests.

Some of the few winter studies on red spruce in the southern Appalachians have focused on winter photosynthesis, which has been reported at or near the compensation point (where gross assimilation = respiration) for the majority of the season. However, it can perform positive photosynthesis during warm winter days, although maximum photosynthesis remains more limited than in the spring (Schaberg 2000; Schaberg et al. 1998). The level to which red spruce responds to these thaw events generally depends more on soil rather than air temperature, as soil temperature dictates the amount of free water available for uptake by the roots (Schwarz et al. 1997). Although this issue of water availability was also explored as a potential contributor to the historic red spruce decline, desiccation due to frozen soils did not appear to be a major factor, further pointing to its injurious dehardening response as the main cause (Perkins et al. 1991). The propensity of spruce to cold-induced foliar injury may also be a factor in its absence from the highest peaks of the southern Appalachians.

As previously mentioned, most winter research in the SSF and NB forest was conducted in the context of air pollution rather than climate change. However, winter

warming and midwinter thaw responses in conifers have been more extensively studied in conifer-dominated ecosystems of the western US, referred to hereafter as western subalpine (WS) forests. Photosynthesis at a WS site in Colorado was found to be more dependent on bole temperature (i.e. frozen vs. liquid water in the trunk) rather than soil temperatures, similarly to findings in the NB forest; no net positive photosynthesis occurred throughout the winter season, even during periods of warm air temperatures (Bowling et al. 2018). In cases where warming reduces the duration of snowpack, the potential benefit of the extended growing seasons appears to be counteracted (and even reversed) by less annual productivity due to a dependence on snowmelt water in spring (Hu et al. 2010). Overall, freeze-thaw cycles early in the spring appear most damaging to alpine and subalpine plants in the process of dehardening, in some cases even reversing the transition (Ensminger et al. 2004; Monson et al. 2005; Venn and Green 2018).

Although the wealth of information on NB and WS conifers is useful for understanding conifer cold hardiness and thaw response, the conditions in these ecosystems do not mirror the exact conditions of the SSF forest, meaning that trees in SSF forests may have habitat-specific adaptations. The key climatic differences between SSF, WS, and NB forests could significantly influence tree response to winter warm periods and, by extension, the respective effects of climate change on the two forest types. Compared to trees in the northern latitudes, trees in the south experience less severe winter temperatures and more frequent midwinter thaw periods. Therefore, it is possible that there may be adaptations in SSF trees to take advantage of these warm periods and that such periods may be more

important to the annual carbon balance of trees in SSF forests than those in NB or WS forests.

Potential adaptations specific to the warmer winters experienced by southern Appalachian conifers could include a greater stomatal sensitivity to warming air temperatures (meaning that needles could recover more quickly in the event of a thaw) or development of specific mechanisms that result in more rapid repair of photosystem II (PSII). PSII is the primary complex that is damaged in photoinhibition, a result of attempted photosynthesis in high light and low temperatures. Trees can also protect themselves from photoinhibition by increasing production of carotenoids, which function as both accessory pigments (capturing wavelengths not absorbed by chlorophyll) and as a crucial tool in avoiding photoinhibition. Xanthophylls (a type of carotenoid) assist in photoprotection by vibrating to dissipate excess light energy as heat in high light/cold temperature situations such as cold sunny days (Demmig-Adams and Adams III 1996). The seasonal downregulation of photosynthesis in conifers in preparation for winter helps to prevent damage to PSII and protect repair mechanisms for PSII when photoinhibitory conditions do occur. This downregulation can take either a rapidly-reversible or slowly-reversible form, and SSF conifers that are not as seasonally cold-stressed could potentially exhibit the former due to a lower risk of extreme freezing events (Verhoeven 2013).

Higher winter temperatures in SSF forests may result in fewer instances of photoinhibition and may also allow for periods of positive photosynthesis, however brief. This could be accomplished when air temperatures are above freezing and water is available (e.g., stored water in the bole), or because warm periods prevent soils from

freezing solid, so root water is available more often during the winter months. A lack of snow cover, already a potential factor in some SSF forests, could also lead to faster warming of soils than when there is a constant snowpack to insulate them (as in WS forests), freeing up more water for uptake. However, rising air and soil temperatures could also increase carbon loss due to increased twig, bole, and root respiration; therefore, it is unclear if winter warming would allow sufficient carbon assimilation to offset respiratory losses. These specific considerations for SSF conifers suggest that climate change may have different effects on trees in the southern Appalachians than in other conifer dominated ecosystems.

If conditions become sufficiently unfavorable due to climate change, trees can respond by migrating to suitable habitat; however, this option is constrained by several factors such as topography (physical barriers to migration), human impact (urban or agricultural areas), or climatic “pockets” of unsuitable conditions between the old and new habitat. For many montane species, climate change triggers a migration to higher elevations or more northerly latitudes, but the migration rate can be much slower at high-elevation ecotones, suggesting that high-elevation communities may be more at risk (Lutz et al. 2013).

The Fraser fir is extremely limited in distribution, and while it appears flexible in its heat tolerance, its potentially weak competitive ability at lower elevations as well as its high seedling mortality rate in exposed areas may prevent it from moving further upslope into new habitat (Berry et al. 2015; Johnson and Smith 2005). Red spruce may be more capable of migrating farther north due to warming, as it is more successful in warmer habitats and is much more common than the Fraser fir. However, the lack of habitats of sufficient elevation

(and therefore mildness) between its current southern occurrence and the northern latitudes may prevent a migration from occurring at all. Southern Appalachian spruce populations may already be completely isolated from northern populations for this reason, reducing the likelihood of a successful migration to milder habitat. Within the SSF forest, red spruce could displace the fir as it moves upslope and the Fraser fir loses its competitive advantage; hardwood tree species could also migrate upslope and potentially displace the conifer populations (Beckage et al. 2008). While many populations of Fraser fir are projected to recover well in higher-elevation sites, population density at lower elevations suggests a high risk of local extinction from lower-lying areas (Kaylor et al. 2016). It seems more likely that, given sufficient warming, the Fraser fir and red spruce could be extirpated altogether from the south.

The precise changes to come in the SSF forest can be difficult to predict, as temperature trends vary depending on scale. Overall, global temperatures are increasing and are projected to exceed current global averages by around 4 °C by the end of the century, although warming by as much as 6 °C is also possible (IPCC 2013). However, temperatures in the southeastern United States have been trending downward in the winter and spring, with average temperatures decreasing since 1958 (Partridge et al. 2018). While the regional cooling effect in the southeast may be tempering the overall warming trend, minimum winter temperatures are rising in the northwest mountains region of North Carolina (Figure 1.4, <https://www.ncdc.noaa.gov/cag/global/time-series>), and 5 of the 10 hottest years on record for the northwest mountains region of North Carolina have occurred since 2010

(Figure 1.5). Even more locally, there is evidence that temperatures are rising on Grandfather Mountain, the field site for this study (Soulé 2011).

Combined, these projections of climate change suggest a variety of potential changes to come in the SSF forest. Disruption of winter season length, intensity, or stability in either direction could have negative effects on the sensitive SSF forest. Therefore, it is critical to understand the current influence of winter on the ecophysiological function of the Fraser fir and red spruce in order to interpret future response to changing winter conditions.

The goal of this thesis is to assess the ecophysiological impact of winter weather on the two dominant trees in the rare southern Appalachian spruce-fir forest, Fraser fir (*Abies fraseri*) and red spruce (*Picea rubens*). This information will become increasingly pertinent as warming due to climate change occurs in this already vulnerable forest.

My thesis research was focused on the physiological activity of native Fraser fir and Red spruce from January to March, 2018, to determine how physiologically active these two species could be during the winter months in the southern Appalachian Mountains. In particular, I was interested in assessing whether these species could achieve positive carbon uptake via photosynthesis during those times when air temperatures rose above freezing, and to determine if they exhibited any photoinhibition when temperatures were below freezing. I used a variety of ecophysiological techniques, such as branchlet gas exchange, needle fluorescence, water relations, and pigment analyses to more fully understand the responses of these trees during the coldest portion of the year. These measurements provide a physiological snapshot of the less-understood wintertime activity in these two

species to which future measurements may be compared as periods of winter warming increase in frequency due to future climate change.

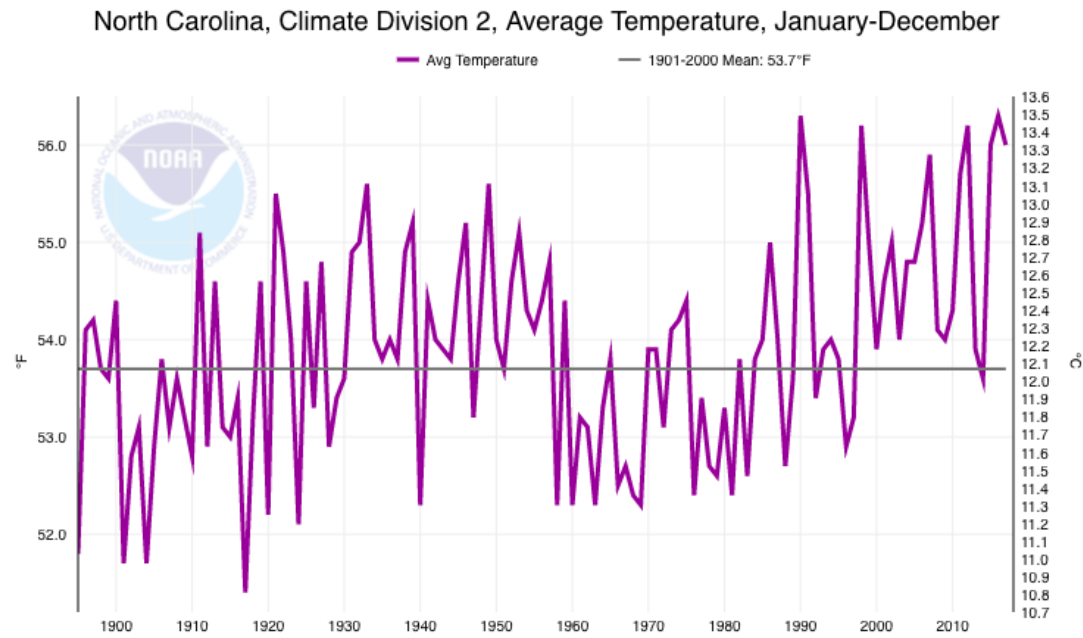


Figure 1.4. Average annual temperature in northwestern NC from 1895-2018. From NOAA NCEI Climate At A Glance.

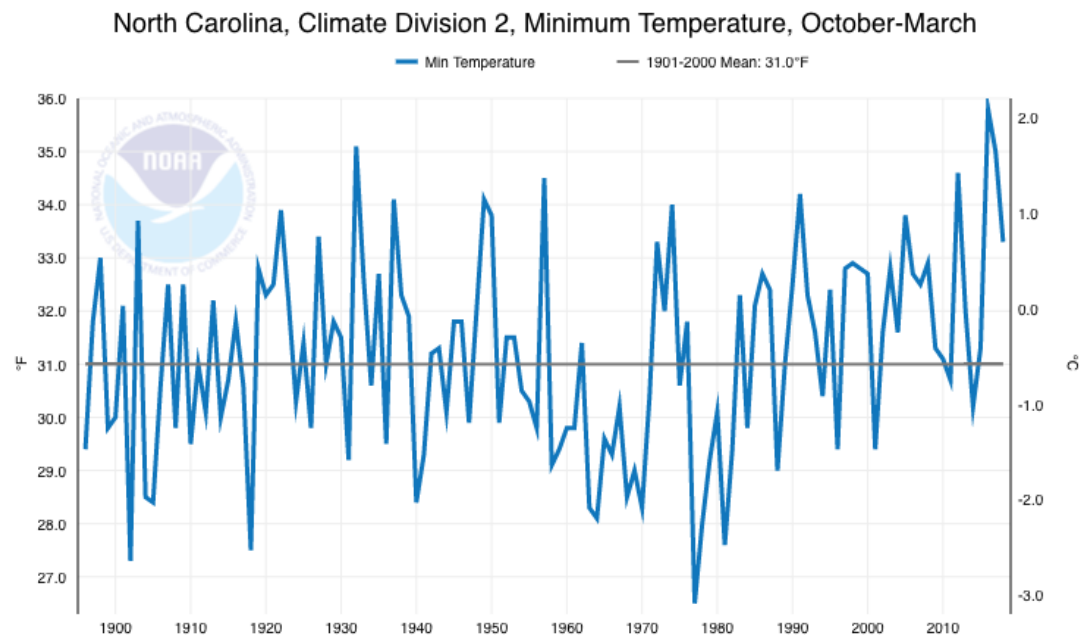


Figure 1.5. Minimum temperature in October-March in northwestern NC from 1895-2018. From NOAA NCEI Climate At A Glance.

Methods

Site Selection

Individuals of red spruce (*Picea rubens*) and Fraser fir (*Abies fraseri*) were sampled from an area adjacent to the lower (northern) end of the Black Rock parking lot on Grandfather Mountain, Linville, NC (36°05'44.3"N, 81°49'45.1"W) at an elevation of 1524 m (Figure 2.1). This east-facing site is within the elevational range of both species and marks the initial transition between mixed spruce-fir forest and the domination of *Abies fraseri* that becomes more pronounced at higher elevations (>1600 m). Trees of both species were growing in close proximity to each other and the farthest tree (a Fraser fir) was less than 50 m away. All trees received ample sunlight during the majority of the day on their south-facing sides, with more shading occurring on their north-facing sides. Because the trees are on protected conservancy land, none had been previously trimmed or altered. Measurements were taken on 5 different days from January to March 2018 (January 25, February 1, February 3, February 22, and March 9).



Figure 2.1. Field site after a riming (frost) event. Sample trees were located near the foreground of this photo.

Gas Exchange

Gas exchange measurements were made on south-facing detached branchlets of ~2.5 cm length (n=3 for each species, 6 trees total) using the Li-6800 portable gas exchange system (Li-Cor, Inc., Lincoln, NE) equipped with the 6 cm² cuvette with LED lighting. These species retain needles for several years, so branchlets with current year (formed the previous summer, i.e., 2017), one-year old (formed 2016), and two-year old (formed 2015) were measured for each tree. Red spruce retain needles for longer than Fraser fir, so branchlets of that species with six year old needles (formed 2011) were also measured. Detached branches were used to save time and also because it was not possible to easily position the instrument near each sample tree.

Preliminary testing indicated an average steady-state gas exchange stability of 15 minutes following cutting of branchlets, and steady-state rates were typically achieved in less than 5 minutes. Water loss from the clipped end of each branchlet was prevented by sealing the end with a small amount of petroleum jelly. The detached branchlet was then placed entirely within the cuvette, which was retrofitted with a stainless steel metal insert that prevented branchlets from falling beneath the thermocouple of the cuvette and which ensured a consistently horizontal orientation of the branchlet (Figure 2.2). Light intensity, relative humidity, and temperature in the cuvette on each day reflected current ambient conditions at that time. Cuvette CO₂ concentrations were kept constant at 400 ppm. Point measurements of assimilation were recorded after a minimum of 2 minutes of steady-state rates, after which the light was turned off and dark respiration recorded following another 2 minutes of stability.

After gas exchange measurements were concluded, branchlets were sealed in small plastic bags and brought back to the lab at Appalachian State University. There, all needles from each branchlet were removed, counted, and placed flat in a standard 8.5" x 11" clear plastic protector. The total projected needle area was obtained by scanning the needles using a Canon 9000F Mark II scanner, obtaining jpg images and using Blackspot open source imaging software (Varma & Osuri 2013) to determine area in cm². The area software was calibrated by measuring the area of disks of known area. Errors in measurement were small enough to be of no consequence. The measured areas for each branchlet were then entered into the Excel data file obtained from the Li-6800 and corrected rates of gas exchange were obtained.

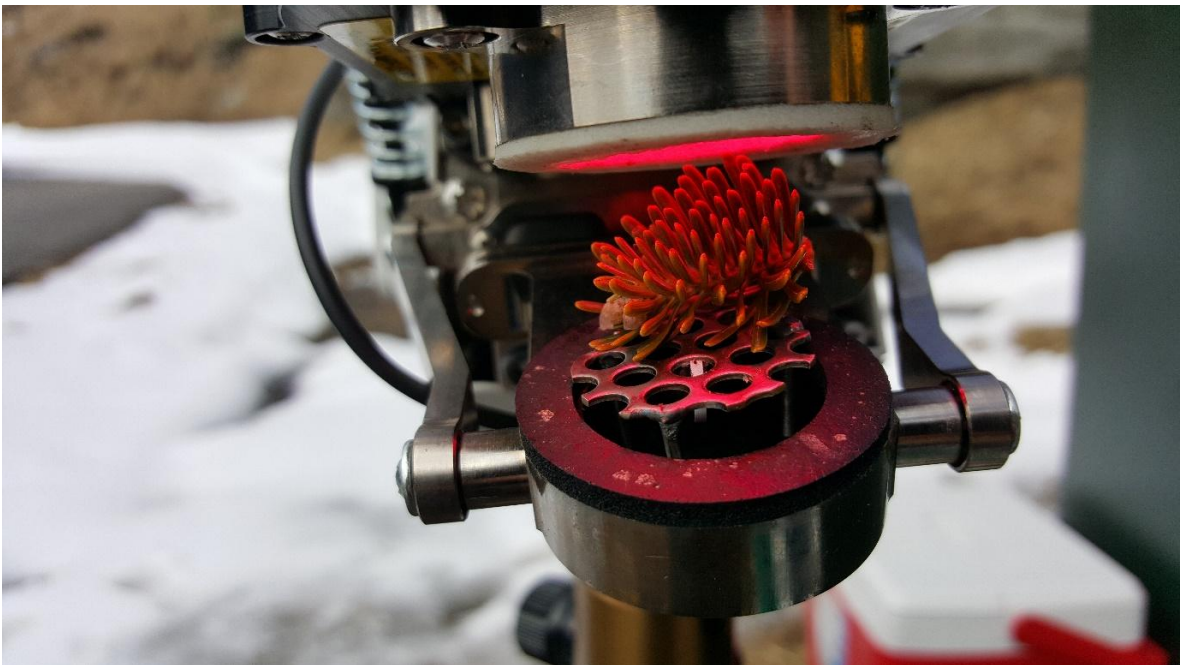


Figure 2.2. A typical *A. fraseri* sample in the Li-6800 cuvette with metal insert.

Water Potential and Chlorophyll Fluorescence Measurements

Water potential measurements were measured on current year branches from the same six trees (one branch per tree) using a PMS Model 600 Scholander Pressure Chamber (Figure 2.3; PMS Instrument Company, Corvallis, Oregon). Measurements were generally taken in late morning to mid-afternoon. Preliminary attempts to measure water potentials proved fruitless when temperatures were below freezing, because the sap in the branchlets was also frozen.

Chlorophyll fluorescence (F_v/F_m) measurements were taken on current year needles using a Hansatech Handy PEA Fluorometer (Hansatech Instruments, United Kingdom). I sampled three branchlets per tree per species. Because the fluorometer is designed for broadleaves, it was necessary to detach up to 10 needles per branchlet and affix them side-by-side onto approximately 1 x 1 cm matte black posterboard squares using double-sided tape. The needles were dark-adapted in the back of a car (to avoid problems with wind) using the fluorometer clips and left to acclimate for 30 minutes before measurements were taken.



Figure 2.3. Scholander pressure chamber setup at field site.

Pigment Concentrations

This portion of the project was led by Zane Sink, who analyzed the pigment concentrations in the needles of both species. His methods and later data are used with permission.

A total of 60 branchlets were collected on February 3, 2018 from trees of both species (n=5 for each species, ten trees total). Needles were collected according to age (Current, First, or Second year) and aspect (North or South-facing). Included in these ten trees were the six trees used in the gas exchange, water potential, and fluorescence measurements.

Following collection, 10 needles were removed from each sample and placed in a drying oven at 30 °C for 24 hours, then weighed in order to calculate dry mass per needle. Another 10 needles were removed from the same samples, placed in small plastic bags, and stored in a freezer at -80 °C for 24 hours. Then, the bags containing the frozen samples were immersed in liquid nitrogen for 20 seconds each; samples were then removed from the bag, and transferred to a mortar and pulverized in liquid nitrogen with a pinch of quartz grinding sand.

After pulverization, the samples were immersed in vials containing 4 mL of N, N dimethyl formamide (DMF) and then refrigerated for 24 hours to allow for pigment extraction. Visual inspection of the samples showed that extraction was essentially complete, with no green pigment remaining in the plant sample material. Absorbances of each sample were made using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan) using quartz cuvettes. The equations of Porra (1989) were used to calculate the

concentrations of chlorophyll *a*, *b*, and total carotenoids, which were expressed on either a projected needle area or dry weight basis.

Statistical Analysis

Gas exchange and pigment concentration measurements were both analyzed using SAS statistical software (SAS, Inc., Cary, NC). Gas exchange measurements over all days were analyzed with a repeated measures ANOVA. There were significant interactions with species in chlorophyll concentrations, but not for carotenoids. Species and needle age were significant ($p < 0.05$) for carotenoids, however, because of the species interactions with chlorophyll, all pigment concentrations were re-analyzed by species using a two-way ANOVA. One outlier point for spruce pigment concentrations was removed from analysis. Water potential and chlorophyll fluorescence measurements were analyzed as two-sample *t*-tests using Sigmaplot (Systat Software, Inc., San Jose, CA). All data met assumptions for parametric analyses, and mean differences were considered statistically significant at $p \leq 0.05$.

Results and Discussion

Weather Patterns and Field Site

Conditions were highly variable during the data collection period, with an anomalously warm day in late February (Table 3.1). Temperatures fluctuated between above and below freezing at the field site throughout the winter, and there was no consistent snowpack during the data collection period. Minimum temperatures were above freezing for 21 of the 74 days between January 1 and March 15, 2018, with more extended periods of subzero temperatures occurring in the middle of this period (Figure 3.2).

With the exception of the first day (which was partly overcast), data collection typically occurred on very sunny days. The field site was also on the southeast-facing side of Grandfather Mountain, meaning that the trees sampled experienced direct sunlight earlier (and in the colder part of mornings) than those on the north-facing side.

Day	Date	Air Temp (°C)	Soil Temp (°C)	Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Relative Humidity (%)
1	1/25	1	---	750-1150 (variable clouds)	50
2	2/1	2	---	1150	20
3	2/3	1	0.3	1150	20
4	2/22	17	7.5	1200	50
5	3/9	-2	-1.5	1500	25

Table 3.1. Daily ambient conditions at field site. The Li-6800 was set to these conditions. CO₂ was kept constant at 400 ppm. Cells marked with “---” denote days for which no soil temperature measurements were taken.

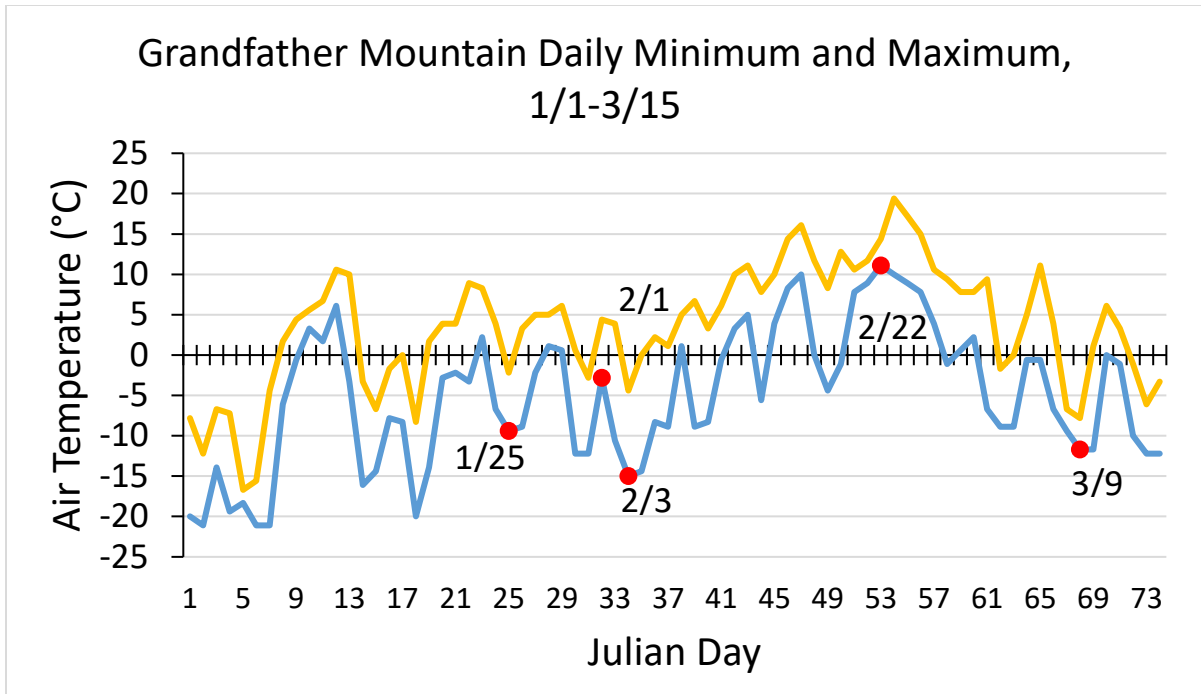


Figure 3.2. Daily minimum (blue) and maximum (yellow) temperatures on Grandfather Mountain. Data collection days are marked in red. From NC CRONOS Database, Station 31356 (<https://climate.ncsu.edu/cronos/?station=313565>)

Gas Exchange

No significant species effect was found for current year photosynthesis on any day (Figure 3.3). One-year-old branches of red spruce exhibited a significantly higher rate of photosynthesis on day 4, the warmest day ($p = 0.01$), but no other days demonstrated a species effect for this age class (Figure 3.4). Dark respiration rate did not differ between spruce and fir in either age class on any day (Figures 3.5 and 3.6). The large amount of dark respiration measured in current year needles on day 5 (the coldest day) is likely erroneous due to very high winds that may have affected the seal of the Li-6800 cuvette. Moreover, the Li-6800 has optimal functioning at temperatures above freezing, so the subzero temperatures on day 5 could have further stressed the instrument.

Net photosynthesis in both species was at or near zero on cold days as expected, especially because soil temperatures were at or below freezing on cold days as well. Both species responded similarly on warm days. The rate of photosynthesis in Fraser fir was about half of its reported summer rates (Cory 2015), but an increase in soil temperatures and a further decrease in snowpack due to warming could increase its ability to respond to warmer air temperatures in the future. The higher rate of photosynthesis in one-year-old needles on day 4 could suggest more rapid dehardening in red spruce than Fraser fir, but the Fraser fir still appeared to be able to take advantage of the warm period to a similar extent, unlike previous studies with the balsam fir and red spruce.

Photosynthetic rates in conifers are highly dependent on the way in which needle area is calculated (Witkowski and Lamont 1991). In my study, areas were calculated as “projected needle area,” or total flat one-sided area of the needles, and rate of carbon assimilation was divided by this area; however, needle arrangement is not flat on the branchlets of either species (Figures 3.3, 3.4), and so self-shading of some needles almost certainly occurs. In addition, the incident radiation on each needle will vary according to the orientation relative to the light source (and on trees in the field according to the sun’s azimuth and solar angle). Thus, expressing A on a projected one-sided needle area most likely results in an underestimation of rates, since this area calculation assumes incident radiation is perpendicular to the needle surface and the same for all needles. However, it would have been too time-consuming to calculate rates in any other manner, and this also standardizes rates to a common basis for both species. Since the Fraser fir has larger

needles than the red spruce, more self-shading may occur, counteracting the potential benefits of larger needle area by resulting in a smaller calculated rate of assimilation.



Figure 3.3. Needle orientation on a typical red spruce branchlet.

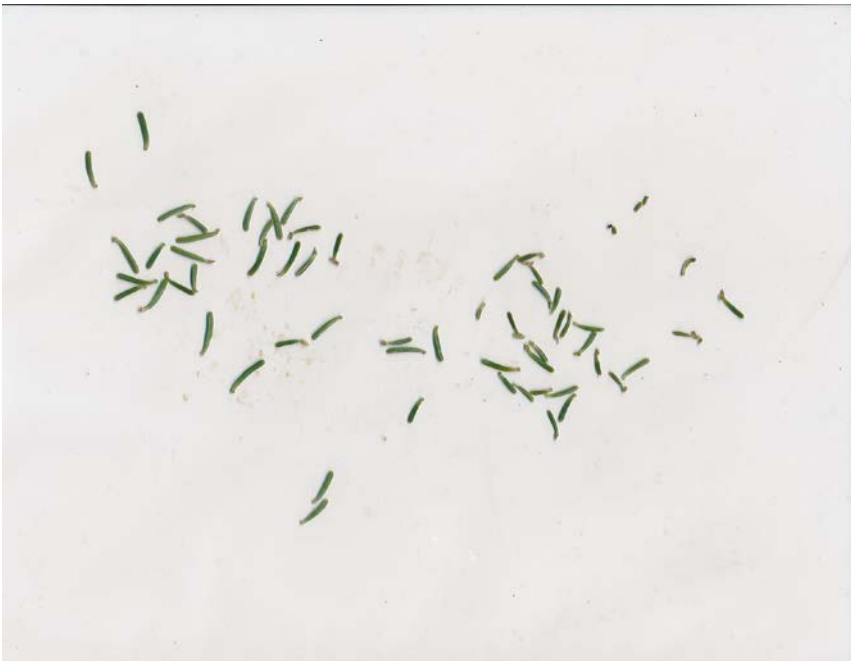


Figure 3.4. Blackspot image of scanned needles used in area calculations.

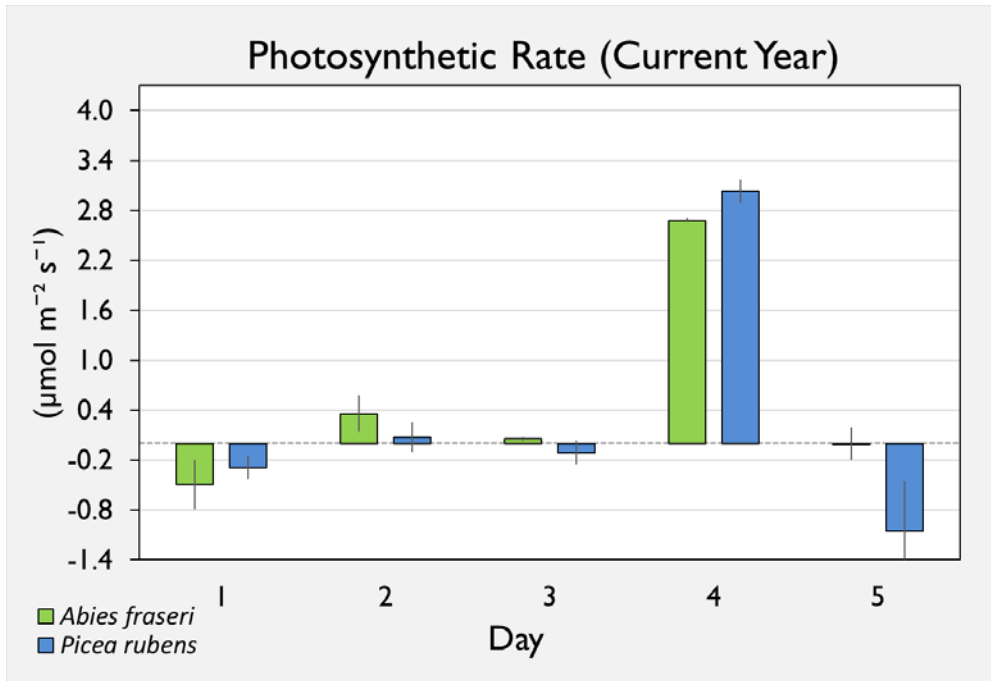


Figure 3.5. Rate of A for current year branchlets. Bars are means \pm se, $n = 3$.

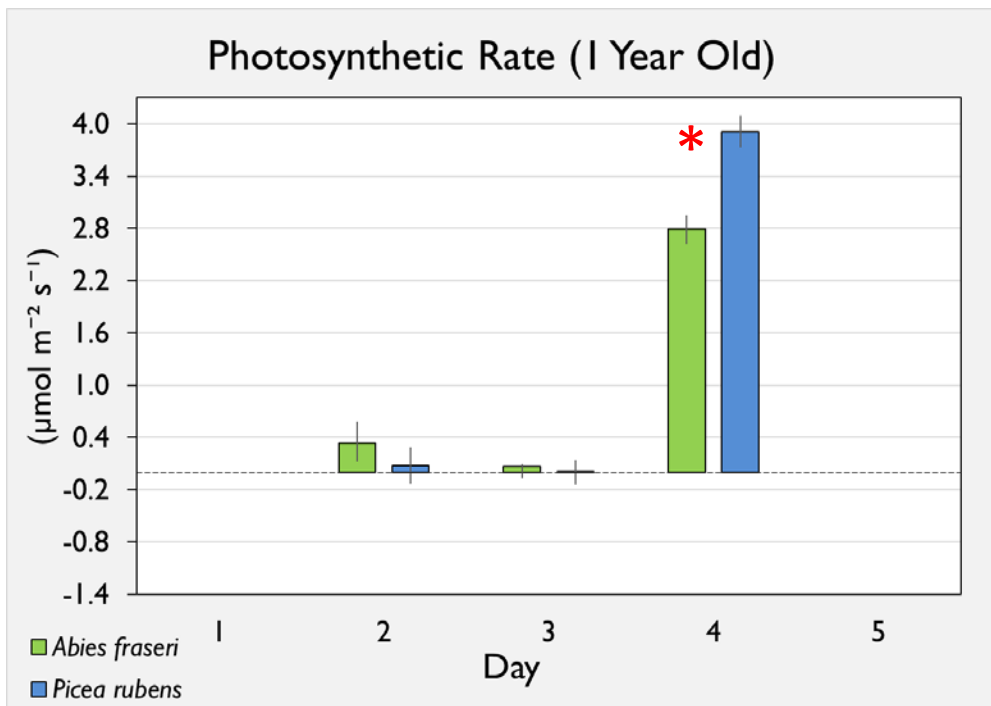


Figure 3.6. Rate of A for one-year-old branchlets. Means marked with an asterisk (*) are significantly different. Blank days indicate no data collection for one-year-old branches for that day. Bars are mean \pm se, $n = 3$.

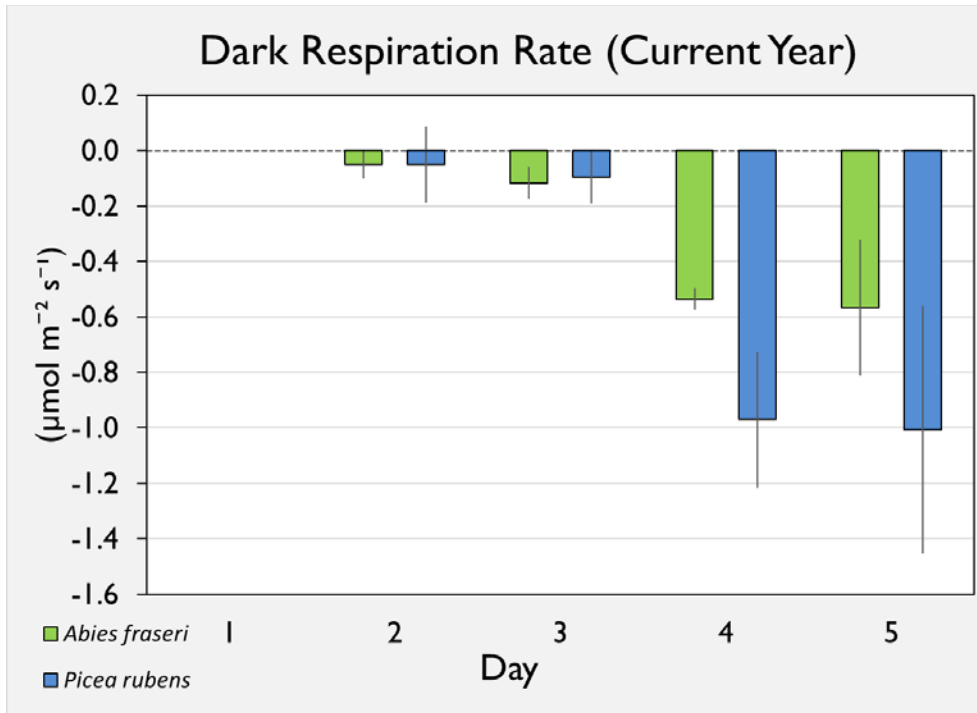


Figure 3.7. Dark respiration rate for current year branchlets. Blank days indicate no measurements for that day. Bars are mean \pm se, $n = 3$.

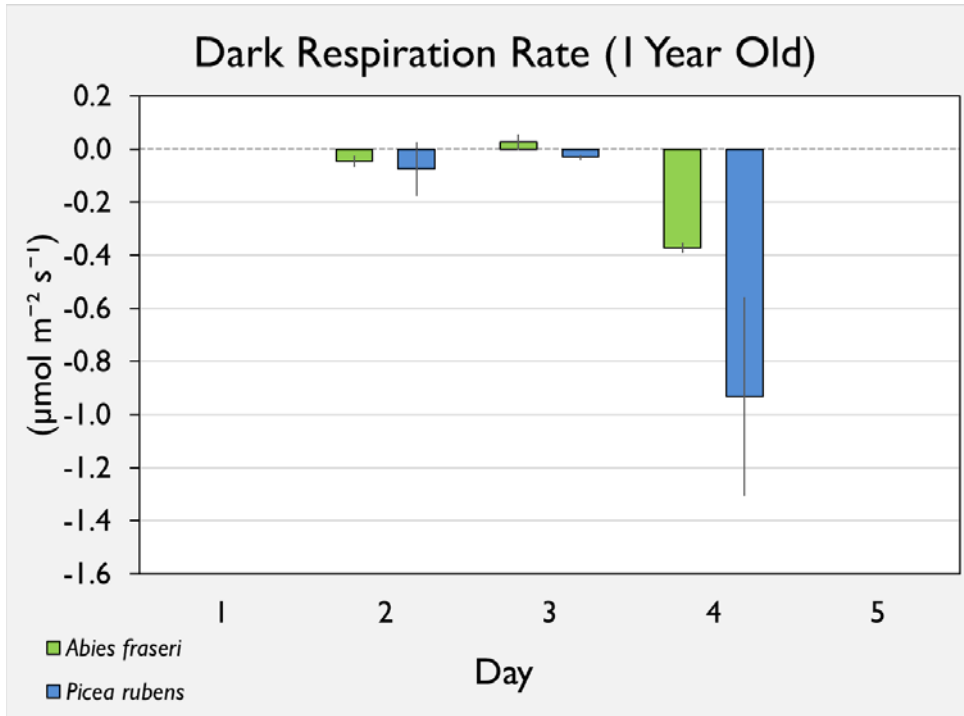


Figure 3.8. Dark respiration rate for one-year-old branches. Blank days indicate no data collection for one-year-old branchlets on that day. Bars are mean \pm se, $n = 3$.

Water Potential and Chlorophyll Fluorescence Measurements

Water potential measurements were significantly lower in the Fraser fir on cold days 2 and 3 ($p < 0.01$) with an overall trend across all days of more negative water potentials in Fraser fir, indicating that Fraser fir may have more open stomata on cold days (Figure 3.7). While transpiration data would help corroborate this potential explanation, transpiration was not analyzed (corrected for area) for the purposes of this thesis due to time constraints. The density of stomata per leaf could be higher in Fraser fir, leading to greater water demand, or the stomatal temperature response of Fraser fir (i.e. the threshold for opening) may allow it to open stomata at lower temperatures than red spruce. The species-specific ratio of xylem (sapwood) to leaf surface area, known as the Huber Value, could also explain the difference in water potentials, but it has not yet been calculated for either species.

Surface soil temperatures (in the top ~15cm of soil) were found to be warmer than those in deep soil, and while Fraser fir has slightly deeper roots than the red spruce, both species have abundant shallow roots; therefore, the competitive advantage of red spruce due to rooting habit is likely quite small (Burns and Honkala 1990). Differences in hydraulic conductivity (K_h , or the amount of water moved through the xylem under known pressure in a given amount of time) could also explain these results. K_h can be affected by several anatomical factors, including xylem conduit diameter; xylem cross sectional area; and density and size of pit pores, which can be safety mechanisms to reduce cavitation (an important consideration in areas like the SSF forest with intermittent freeze-thaw cycles) (Pittermann and Sperry 2006; Sperry and Sullivan 1992).

Chlorophyll fluorescence did not differ between spruce and fir, but it did increase significantly with air temperature ($p = 0.04$) (Fig. 3.8). This indicates less stress, i.e. less photoinhibition, on warm days. Since photoinhibition in the winter occurs during periods of high light and low temperature, rising temperatures were expected to decrease photoinhibitory stress and therefore increase chlorophyll fluorescence. The similar F_v/F_m ratios coupled with pigment results (discussed below) may also shed light on differences in xanthophyll cycle activity as a means of photoprotection.

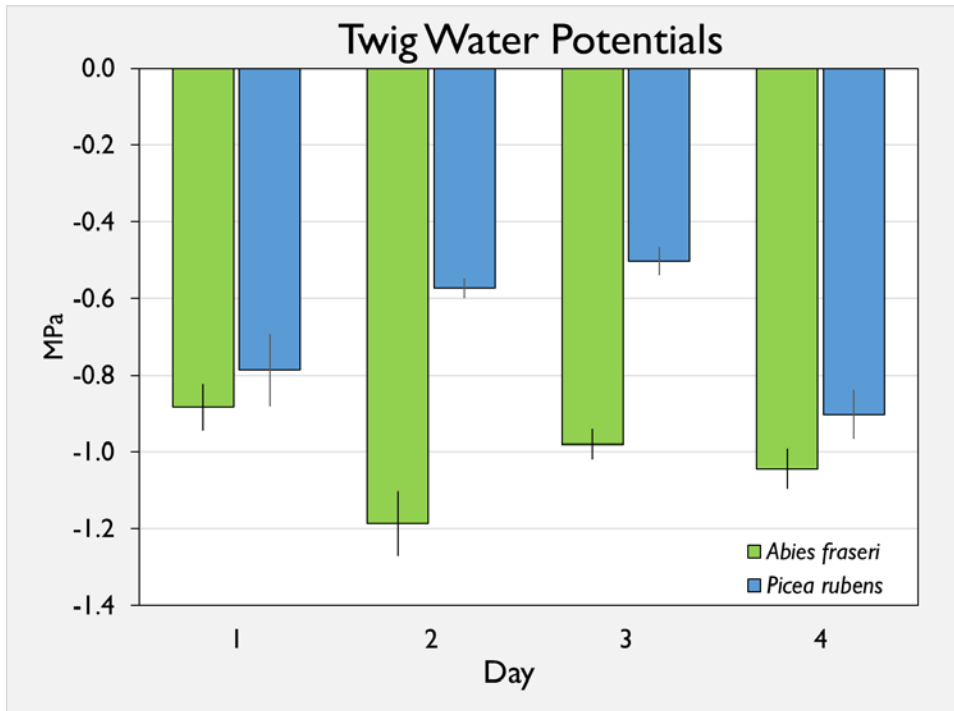


Figure 3.9. Water potential measurements for days 1-4. Means marked with an asterisk (*) are significantly different. Bars are mean \pm se, n = 3.

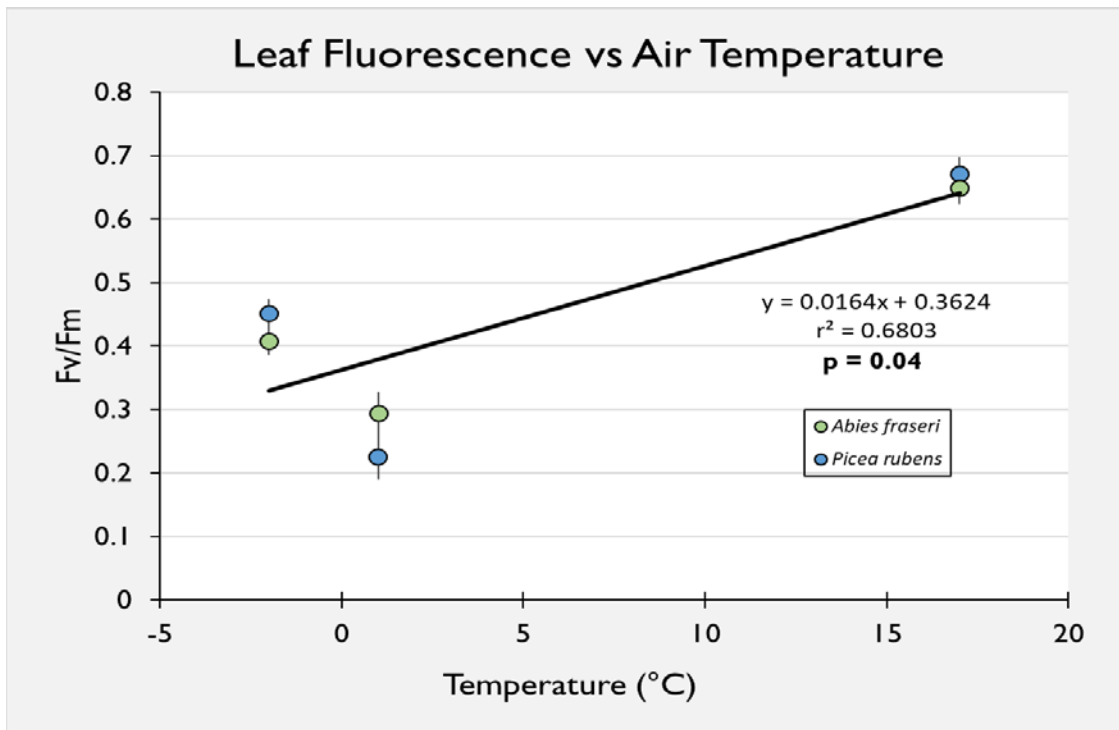


Figure 3.10. Chlorophyll fluorescence for days 2-4, current year needles only. Points are mean \pm se, n = 3.

Pigment Concentrations

Total chlorophyll (a and b) and carotenoids were significantly higher in current year needles of Fraser fir than those of red spruce ($p < 0.001$) (Fig. 3.8). Total chlorophyll and carotenoid concentrations declined with needle age in both Fraser fir ($p < 0.05$) and red spruce ($p < 0.01$), although the magnitude of the trend was less pronounced in red spruce (Fig. 3.12, 3.13). Aspect (north or south-facing) did not have an effect on pigment concentrations in either species ($p > 0.05$).

It is curious that the two species have fairly similar rates of photosynthesis despite vastly different pigment concentrations, suggesting several possible explanations. The difference may be anatomical: despite higher concentrations of chlorophyll, the Fraser fir may be constrained by internal diffusional limits on CO_2 movement: that is, CO_2 cannot diffuse quickly enough within the tissues to result in higher rates of photosynthesis, thereby offsetting the benefit of extra chlorophyll. These results could also be explained by differences in amounts and/or activation state of the carboxylation enzyme RUBISCO. The shallower roots of red spruce than those of the Fraser fir (Burns and Honkala 1990) may contribute to different uptake of nitrogen (N), an important nutrient in the formation of chlorophyll pigments and RUBISCO. If deeper roots can obtain more N in the soil, then more photosynthetic pigment can be produced per needle, potentially explaining the significantly larger per-needle amounts of total chlorophyll and carotenoids in the Fraser fir with similar rates of photosynthesis to the red spruce, which may allocate less N per needle due to lower N uptake. Moreover, many needles on the red spruce trees during the winter season

were observed to be chlorotic (yellow), possibly indicating degradation of chlorophyll due to photoinhibition (Godde and Dennehl 1994).

Another potential (and more probable) explanation is a lack of dehardening in Fraser fir that constrains photosynthetic rate during warm periods despite the larger concentrations of chlorophyll. Fraser fir may downregulate photosynthesis more aggressively in the winter, resulting in a relative inability to deharden fast enough to take advantage of warming, as occurs in balsam fir (Strimbeck et al. 1995). Moreover, the pigment differences coupled with the similar rates of fluorescence seen in these two species indicate that there may be more non-photochemical quenching in Fraser fir than in red spruce (i.e. the xanthophyll cycle may be more active in the fir) (Demmig-Adams and Adams III 1996). If Fraser fir deharden more slowly, and perhaps later in the season when the weather begins to warm up more consistently, that would leave it with a shorter growing season in which to obtain sufficient carbon. Thus, the higher pigment concentration may allow higher rates of assimilation in the warmer months, and make up for the shorter growing season at higher elevations, where this species is dominant.

The decline in pigment concentrations with increasing needle age may provide a non-hydraulic explanation for significant declines in photosynthesis with needle age seen in Fraser fir (Cory 2015). While photosynthesis and specific leaf area (cm^2/g dry mass) decline with tree (not needle) age in red spruce, stomatal conductance and internal CO_2 do not differ with age, meaning that age does not appear to affect stomatal density or function in this species (Day et al. 2001).

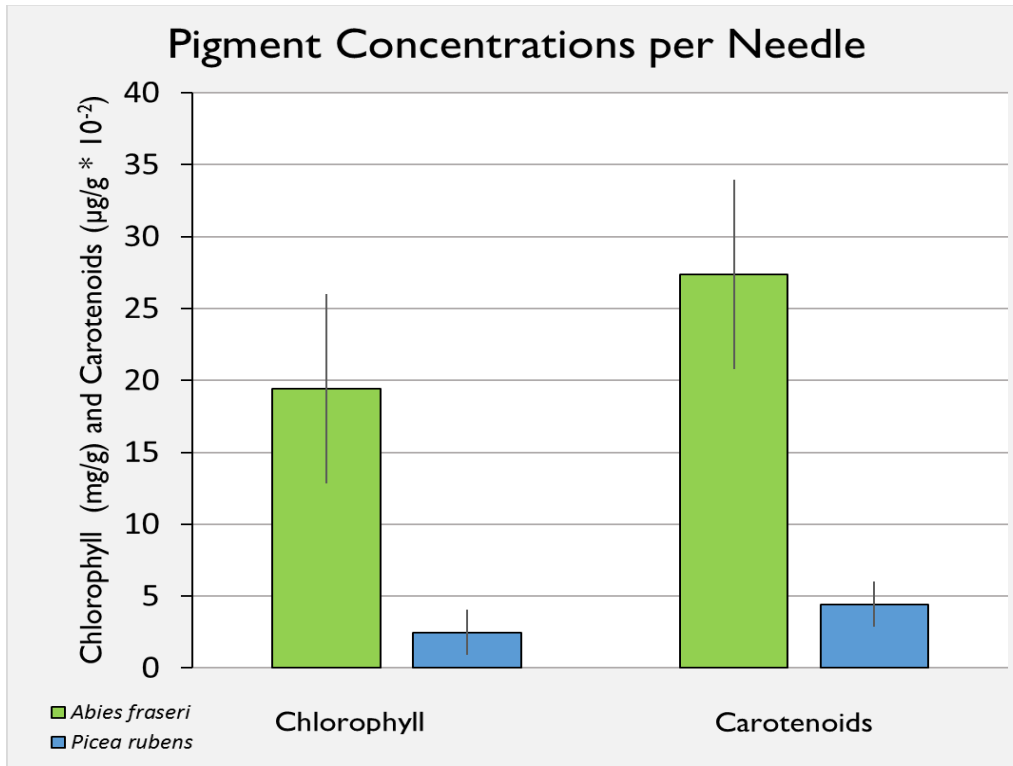


Figure 3.11. Pigment concentrations of total chlorophyll *a* and *b* and carotenoids. Means marked with an asterisk (*) are significantly different. Bars are mean \pm se, $n = 3$.

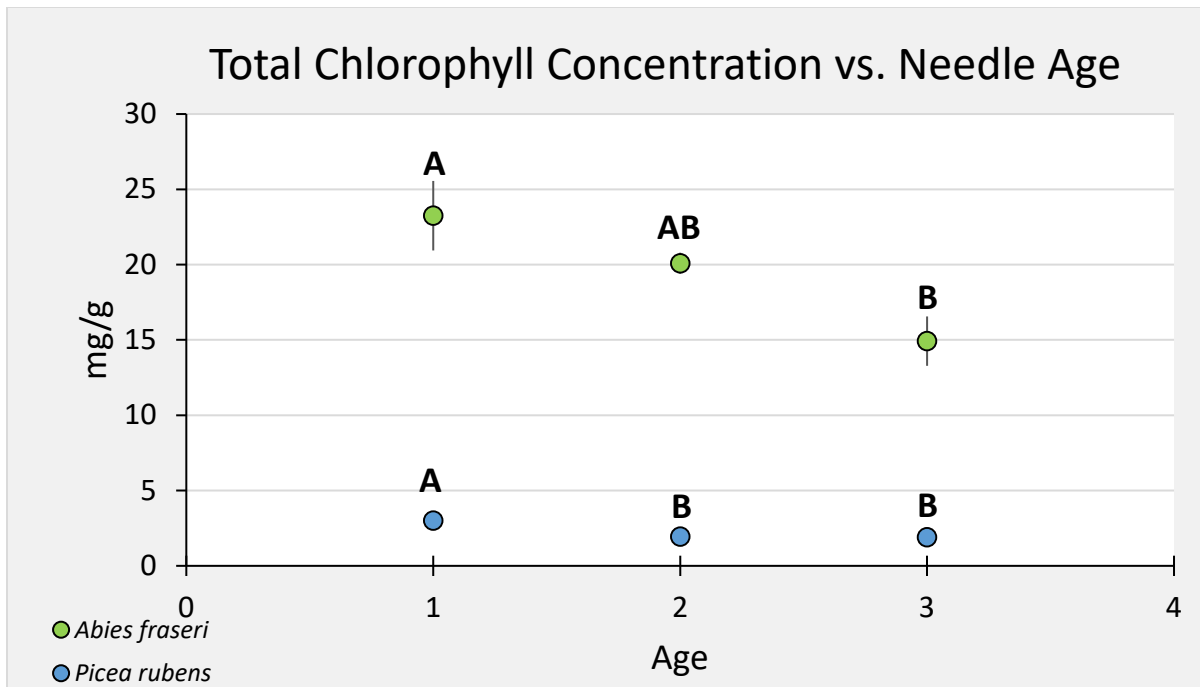


Figure 3.12. Total chlorophyll (*a* and *b*) and carotenoid concentration (mg per g dry weight) as a function of needle age. Means within a species that share a letter are not significantly different. Symbols are mean \pm se, $n = 3$.

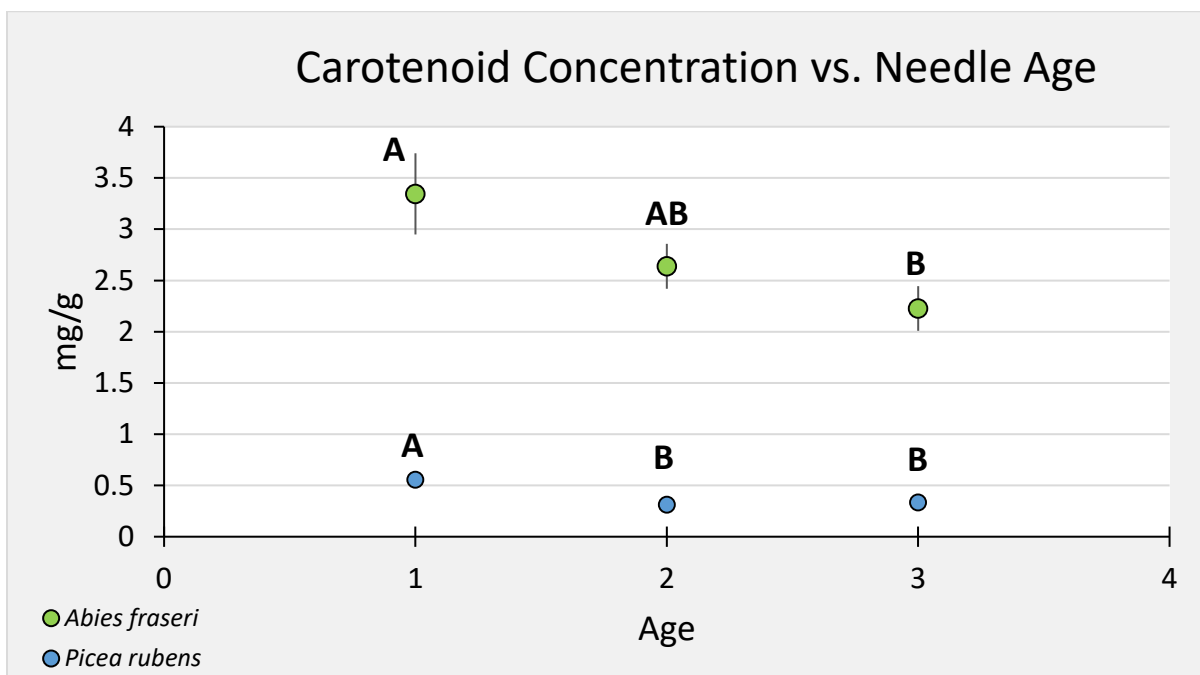


Figure 3.13. Total carotenoid concentration (mg per g dry weight) as a function of needle age. Means within a species that share a letter are not significantly different. Symbols are mean \pm se, $n = 3$.

Future Directions

The results of this study indicate a need for further exploration of the factors that may drive the differential distributions of Fraser fir and red spruce. More frequent measurement days, preferably at regular intervals, are recommended for a more robust analysis, assuming the weather cooperates. Further comparative study of red spruce and Fraser fir should include measurement of the dehardening response in thaw periods for multiple consecutive days and through subsequent frost periods. Due to the warmer annual temperatures in the southern US, it is crucial to understand whether a thaw response disparity similar to that of balsam fir and red spruce exists in the SSF forest (Strimbeck et al. 1995).

Other knowledge gaps that warrant further study are comparison of hydraulic conductivity (K_h ; the amount of water moved through the xylem under known pressure in a given amount of time) as a potential explanation of water potential trends seen in this study; correlation of physiological data with snowpack depth, and more in-depth analyses of needle concentrations of pigments, carbohydrates, and proteins associated with downregulation of photosynthesis in the winter. Xanthophyll cycle regulation in anticipation of winter (as well as during midwinter thaws) could be a particularly interesting area of comparative study, as it would shed light on the potentially different photoprotective measures taken by spruce and fir.

Since the aspect of the field site itself may have been a factor in the results of this study, future studies could include trees on both north and south faces of the mountain. As previously mentioned, trees on the north and west facing sides of the mountain do not

receive early morning sun, which results in a different diurnal cycle of photosynthesis and water relations than that of trees on the south and/or east-facing side. The highest intensity of light on the N and W sides would also occur in the afternoon, when temperatures are higher, meaning that less photoinhibition may occur throughout the winter.

A study of these trees through an entire seasonal cycle may illuminate physiological differences during the winter-to-summer and summer-to-winter transition periods. Multiple SSF sites could be used in a larger-scale study as well, as SSF forests are isolated from one another as well as at different elevations (Grandfather Mountain is at the lower end of the SSF elevational gradient), meaning that they may exhibit different levels of physiological response to winter.

Rising atmospheric CO₂ concentrations may affect winter response on days favorable for photosynthesis (sufficient water availability and warmer temperatures, as in a midwinter thaw), when stomata are open and are able to take up excess CO₂. The stomata will most likely remain closed in subzero temperatures, cutting off the major pathway of carbon uptake in freezing periods and counteracting the possible benefit of increased CO₂. However, carbon can still be assimilated by other pathways. Dissolved CO₂ in the xylem can be transported to branchlets/needles and used in photosynthesis (Teskey 2008). Furthermore, many trees have a layer of chlorophyll beneath the bark of young twigs and stems that is capable of performing photosynthesis and appears to be especially useful in photoinhibitory conditions (Pfan and Aschan 2001). Potential winter response in the presence of elevated CO₂ and cold temperatures could be measured by developing growth chamber studies or creating CO₂ curves for trees in the wild.

Growth chamber or garden studies of seedling response to controlled midwinter warming periods could also inform projections of future response to more frequent freeze-thaw events. Manipulations could include winter lengths (e.g. long vs. short); midwinter thaws in early, mid, and late winter, since regulation can vary throughout the winter (Ensminger et al. 2004); and soil temperature changes to separate its effect on winter photosynthesis. In general, more controlled experiments are recommended for a deeper understanding of single variables affecting winter physiology that cannot be easily isolated in the wild; results of controlled seedling studies in particular could suggest how regeneration rates could change in a warming climate. These results can be compared with studies of wild trees as well as those in farm and silvicultural settings to develop a projection of the respective environmental impacts on these two conifers in different settings.

Conclusion

While the impacts of climate change in all seasons have not been fully elucidated for the southern Appalachian spruce-fir forest, it is apparent that certain factors currently influencing its function—such as frequency of cloud immersion and subzero temperatures, snowpack depth and consistency, soil temperatures, and midwinter thaw occurrences— are at risk of being significantly altered. Future winters may be warmer, with less frequent cloud immersion and subzero temperatures, snowpack depth and persistence may be reduced, and soil temperatures and the frequency of midwinter thaws may increase. . Fraser fir and red spruce may have different strategies for avoiding photoinhibition, frost injury, and water stress, meaning that the current composition of the spruce-fir forest may change depending on future conditions. In the farm setting, Fraser fir appears to have a good prognosis for survival under current projections for future warming, but wild populations may still be at risk due to more complex competitive factors and sensitivities in the wild (Cory 2015, Stevens 2017). This study serves as an initial exploration of the least-understood season in the SSF forest. A deeper understanding of this rare ecosystem in all seasons will help inform future silvicultural, commercial, and conservation efforts for these two iconic southern Appalachian conifers.

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